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SURVIVAL OF SOIL MICRO-ORGANISMS UNDER PROLONGED DESICCATION

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ABSTRACT

Survival of soil micro-organisms stressed by prolonged desiccation was determined in the laboratory. Pure cultures of bacteria isolated from soil were subjected to the same stress. It was found that both physiological age of culture and water activity of the medium affected the death rate of cells. Possible mechanisms are postulated.

INTRODUCTION

Micro-organisms differ in their abilities to take up water from the soil (Griffin 1963, Konyeas 1964). It is generally assumed that low soil water potentials, as in dry soil, inhibit growth and reproduction of micro-organisms (Cook and Papendick 1970). Micro-organisms, however, do survive long periods in dry soils, and this has been attributed to the formation of resistant structures or forms. Resistant structures such as endospores and cysts of bacteria, conidia of actinomycetes, and chlamydospores, sclenotia, rhizomorphs and conidia of fungi are well known. But all these structures are dormant and wait for favourable conditions before active metabolism can take place. There are, however, certain bacteria which remain viable when desiccated, without forming specialised survival structures.

Different aspects of the problem have been examined, but studies of microbial survival in such environments and explanations for the persistence of these long-lived species are few. In this study three experiments were set up to investigate:

1. death rates of different physiological groups of soil micro-organisms under induced desiccation
2. effect of physiological age of selected bacteria on their rate of survival under induced desiccation
3. effect of pre-treatment of pure cultures of bacteria, at different water activities, on their survival under induced desiccation.

MATERIALS AND METHODS

The experimental methods used were a modification of those described by Chen and Alexander (1973).

Silty loam soil was sampled from a flower bed by the Department of Botany, University of Canterbury. This soil

contained 0.26% nitrogen, 2.7% carbon and pH 6.4 (Waid pers. comm.). The soil was sieved through 2.0 mm mesh immediately after sampling. Samples (10 g) of soil were placed in sterile glass Petri dishes (70 mm diam.). Fifteen replicates of sampled soil were incubated at 30°C in an improvised desiccator jar with silica gel as drying agent. The jar was opened daily to renew the drying agent as well as to reventilate the jar. Samples were removed from the jar at selected intervals of 2, 7, 15, 30 and 60 days so that survival of different physiological groups of bacteria could be determined.

1. TOTAL VIABLE COUNTS

Viable micro-organisms in the soil were counted by a standard dilution plate technique (Parkinson, Gray and William 1971) and differentiated on different media. The four agar media used were:

(i) soil extract agar amended with 0.2% yeast extract (Holding 1960)
(ii) Thornton's agar medium (Thornton 1922)
(iii) crystal violet agar (Holding 1954)
(iv) sodium azide agar (Lichstein and Soule 1944)

Soil extract agar favours the growth of most soil bacteria and thus the overall bacterial populations are counted. Thornton's agar medium represses most spreading colonies which might interfere with the counts. Crystal violet agar permits the growth of Gram-negative bacteria but supresses Gram-positive bacteria. Gram-negative bacteria are believed to be the predominant bacteria in soil (Holding 1960, Clark 1967). Sodium azide agar isolates Gram-positive bacteria from the soil. Triplicate plates for each agar were incubated in the dark at 30°C. The plates were examined regularly and final counts recorded after 14 days. The micro-organisms were classified and recorded using methods of Taylor and Lochhead (1938) and Harrigan and McCane (1966).

2. PHYSIOLOGICAL AGE OF BACTERIA

Pure cultures of drought-susceptible and drought-resistant strains of bacteria were isolated by standard isolation techniques (Parkinson, Gray and William 1971, Aaronson 1970) during desiccation. Those strains which died after 1-4 days of desiccation were considered drought-susceptible, and those surviving at least 15 days desiccation were considered drought-resistant.

Two drought-resistant rod-shaped strains of bacteria were chosen: a Gram-negative rod (RS2) and a Gram-positive rod (RS6). These were grown separately in 500 ml Erlenmeyer flasks containing 200 ml nutrient broth. The cultures were placed on a rotary shaker and incubated at 25°C. After selected intervals of 17h, 65 h, 6 days, and 10 days, the cultures were centrifuged to collect the bacterial cells. These bacterial cells were washed three times in 0.10 M sodium phosphate buffer adjusted to pH 7.0 and then the cells were suspended in buffer solution. Bacteria in suspension were counted by plating out dilutions in nutrient agar. 2 ml of suspension was then added to 10 g sterile quartz sand contained in Petri dishes (70 mm diam.). The cultural strains in the Petri dishes were then subjected to the same conditions of desiccation as

described earlier. On alternate days two Petri dishes of each cultural age were withdrawn from the desiccator jar and the survival rate of bacteria in the quartz sand assessed on triplicate plates of nutrient agar.

3. PRE-TREATMENT WITH DIFFERENT WATER ACTIVITIES

Both brain-heart infusion medium, and nutrient broth containing a 5:3:2 mixture of NaCl, KCl and Na_2SO_4 were used for pretreating bacteria. Media were adjusted to different water activity (A_w) values derived from Scott (1953). Before the experiment, any loss of water was checked by weighing the prepared solutions. A single strain of isolated bacteria RS6 was then inoculated into the medium. After 7 days incubation at 27°C the turbidity of the broth was recorded. Cells grown in solutions of 0.99 A_w and 0.98 A_w were collected by centrifuging, washed three times and grown on quartz sand, and subjected to the same conditions of desiccation and sampling as in procedure 2 above.

RESULTS

1. TOTAL VIABLE COUNTS

Relationships between various groups of micro-organisms under prolonged desiccation are shown in Fig. 1 and Tables 1, 2 and 3. In all three media, numbers of soil micro-organisms significantly decreased when cultures were subjected to decreased water potentials (Fig. 1). This graph also shows the selective effects of the different media for various physiological groups of micro-organisms. Survival rates of different physiological groups of micro-organisms were inconsistent, indicating different susceptibilities to drought. Within classified physiological groups in the tables, however, populations of different strains of organisms were not differentiated. This may give misleading population counts within the groups. The results show that only a few strains

TABLE 1. SURVIVAL OF SOIL MICRO-ORGANISMS DESICCATED OVER SILICA GEL AND PLATED OUT IN SOIL-EXTRACT AGAR AMENDED WITH YEAST EXTRACT Counts are 10^3/g soil, and are followed by % survival in parentheses.

DAYS	0	2	7	15	30	60
Total counts	28000 (100)	23700 (85)	14400 (51)	17800 (64)	13100 (47)	8000 (29)
Actinomycetes	17200 (100)	16900 (98)	9500 (55)	12400 (72)	8900 (52)	6108 (36)
Gram-negative bacteria	5700 (100)	3000 (53)	2100 (37)	2500 (44)	1900 (33)	920 (16)
Gram-positive bacteria	4000 (100)	2800 (70)	1900 (48)	2100 (53)	1700 (43)	876 (22)
Cocci	1900 (100)	1000 (53)	900 (47)	800 (42)	600 (32)	96 (5)

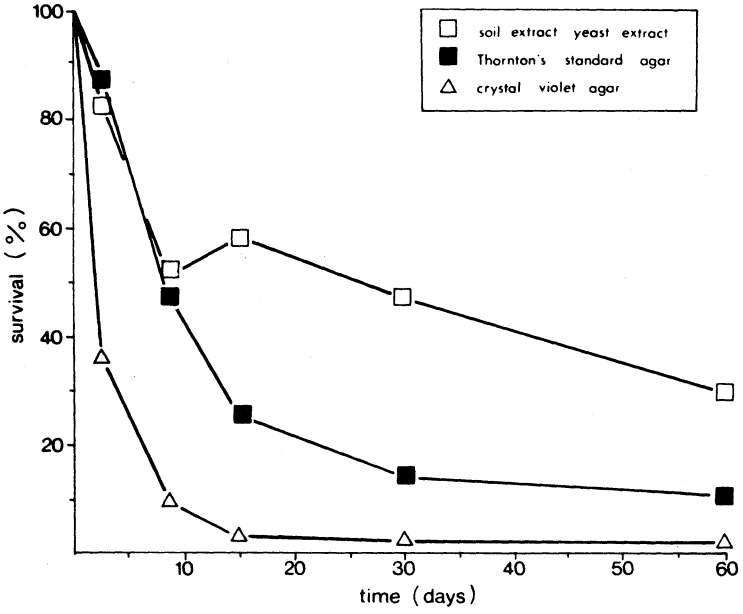

Fig. 1. Percent survival of soil micro-organisms desiccated above silica gel and grown on three different media.

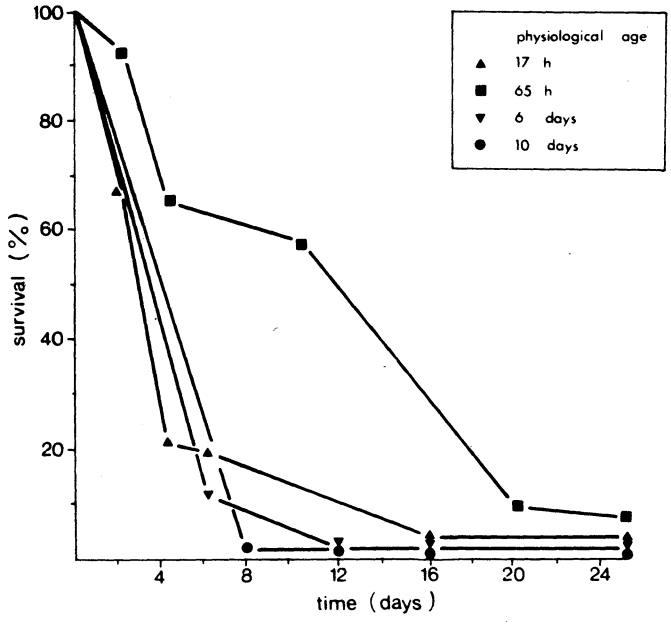

Fig. 2. Percent survival of Gram-negative bacteria RS2 of different physiological age.

TABLE 2. SURVIVAL OF SOIL MICRO-ORGANISMS DESICCATED OVER SILICA GEL AND PLATED OUT IN THORNTON'S AGAR MEDIUM
Counts are 10^3/g soil, and are followed by % survival in parentheses.

DAYS	0	2	7	15	30	60
Total counts	24200 (100)	21200 (88)	12000 (50)	6100 (25)	2970 (12)	2450 (10)
Actinomycetes	18600 (100)	16800 (90)	9300 (50)	4100 (22)	2150 (12)	2005 (11)
Gram-negative bacteria	3900 (100)	2250 (58)	1700 (44)	1385 (36)	643 (17)	356 (9)
Gram-positive bacteria	1100 (100)	1560 (>100)	650 (59)	450 (41)	117 (11)	77 (7)
Cocci	600 (100)	590 (98)	340 (57)	165 (28)	60 (10)	12 (2)

TABLE 3. SURVIVAL OF SOIL MICRO-ORGANISMS DESICCATED OVER SILICA GEL AND PLATED OUT IN CRYSTAL VIOLET AGAR
Counts are 10^3/g soil, and are followed by % survival in parentheses.

DAYS	0	2	7	15	30	60
Total counts	3000 (100)	1100 (36.7)	220 (7.3)	31 (1.0)	20.7 (0.7)	9 (0.3)
Actinomycetes	447 (100)	100 (22.4)	21 (4.7)	2 (0.5)	1 (0.2)	0.05 (<0.1)
Gram-negative bacteria	2181 (100)	912 (41.8)	186 (8.5)	25 (1.2)	18.7 (0.9)	8.88 (0.4)
Cocci	372 (100)	88 (23.7)	13 (3.5)	4 (1.1)	1 (0.3)	0.07 (0.2)

of these bacteria can grow and reproduce under low soil water potentials. It must also be noted that micro-organisms capable of growth at low water potentials may escape the competition and antagonism of those organisms not capable of growth at such potentials.

2. PHYSIOLOGICAL AGE OF BACTERIA

For this experiment pure culture strains which could not grow from a dormant structure were selected by endospore staining and microscope examination.

A Gram-negative rod bacteria (RS2) with a physiological age of 65 h was most resistant to desiccation (Fig. 2). For the Gram-positive rod bacteria (RS6) the 6 day-old culture was most resistant (Fig. 3). Bacteria of both strains, however, decreased in all treatments when desiccated. This was consistent with results from experiment 1.

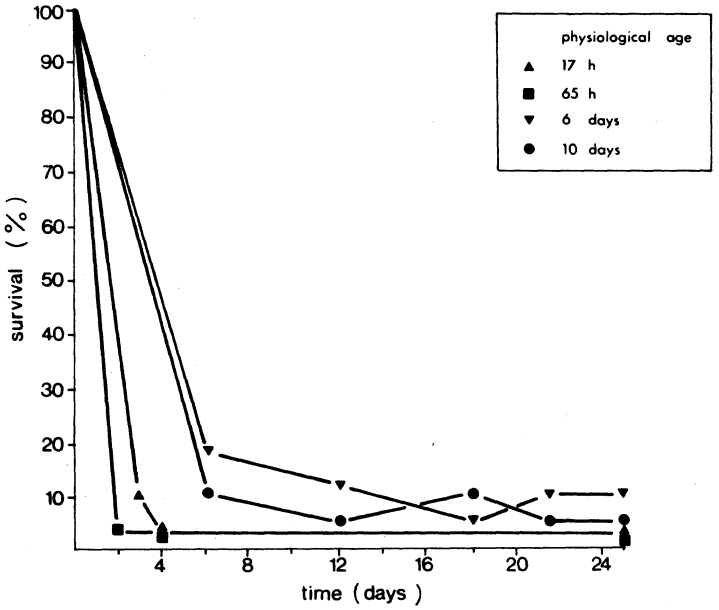

Fig. 3. Percent survival of Gram-positive bacteria RS6 of different physiological age.

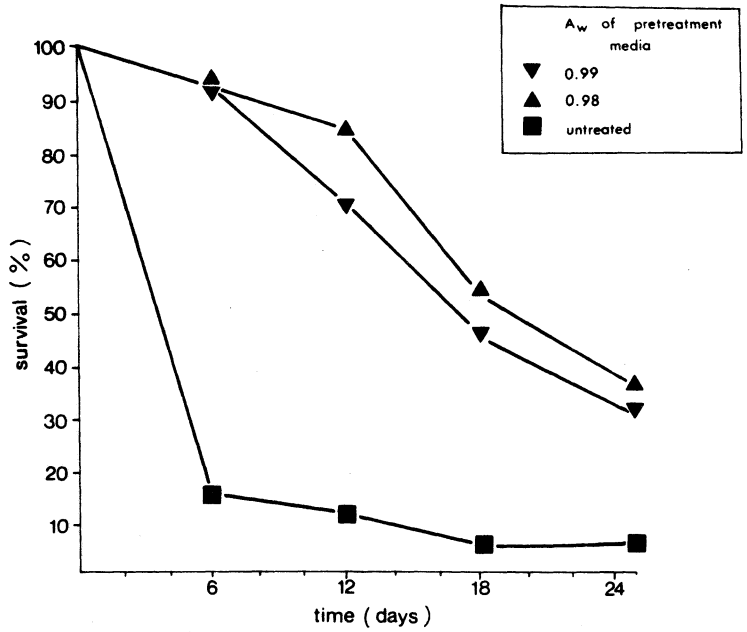

Fig. 4. Percent survival of 7-day old culture of RS6 after pretreatment with media of different water activities (A_w).

3. PRE-TREATMENT WITH DIFFERENT WATER ACTIVITIES

24 hour old culture of RS6 was inoculated into media of different water activities ranging from 0.99 A_w to 0.88 A_w. The results after 7 days and 14 days were recorded (Table 4).

TABLE 4. SURVIVAL OF BACTERIUM RS6 GROWN IN DIFFERENT WATER ACTIVITIES, AFTER 7 AND 14 DAYS IN AMENDED NUTRIENT BROTH AND BRAIN-HEART INFUSION MEDIUM. + growth; - no growth; -* flake formation on top of medium. A_w not checked for the 14-day period.

	AMENDED NUTRIENT BROTH		BRAIN-HEART INFUSION MEDIUM	
A_w	7 days	14 days	7 days	14 days
0.995	-	+		
0.990	-	+	+	+
0.980	-	+	+	+
0.970	-	-*	-	+
0.960	-	-	-	-*
0.940	-	-	-	-
0.920	-	-	-	-
0.900	-	-	-	-
0.880	-	-	-	-
0.860	-	-	-	-

During the first week of incubation bacteria RS6 adapted to grow in the brain-heart infusion down to 0.98 A_w. In the second week, it adjusted to grow at 0.97 A_w. The same inoculated culture in the amended nutrient broth, however, did not show any activity until the second week of incubation. This means that either the two media had a differential effect on the growth of bacteria, or the majority of cells burst upon addition of inoculum to the medium so that the population had to build up from a small number of surviving cells. Pre-treated cells at 0.99 A_w and 0.98 A_w were used for the experiment.

Effects of induced desiccation on pre-treated bacteria and normal untreated cells were compared (Fig. 4). After a period of 25 days, 6.0% of untreated cells remained, compared with 29.0% and 37.0% respectively for bacteria pre-treated at 0.99 A_w and 0.98 A_w. Hence, pre-treatment of a parent cell population clearly affects subsequent cell longevity in increased salt concentrations.

DISCUSSION

Skinner (1968) noted the remarkable ability of micro-organisms to become modified in many different ways under adverse environmental conditions. Ingram (1957) suggested that micro-organisms resist high solute concentration in their environment by increasing their internal solute concentration to approximate that of the medium in which they grow. Cook and Papendick (1970) contended that:

ψ external environment = ψ cell turgor + ψ cell osmotics,

where ψ is the water potential. Thus as soil dries, cells tend to lose water to the environment and simultaneously

increase internal osmotics until equilibrium is reached. In this paper, the results of experiments 1 and 3 imply an ability of certain micro-organisms to perform such a process. It has been suggested (Chen and Alexander 1973) that increased internal osmotic tension is one of the factors involved in the mechanism of drought resistance.

Skaliy and Eagon (1972) worked with *Pseudomonas aeruginosa* and found that a 7-day culture exhibited the greatest resistance to desiccation. In the present work physiological age of bacteria is shown to be another factor in drought resistance in both Gram-negative and Gram positive rods. The explanation for the effect of physiological age of bacteria on resistance to desiccation is probably similar to that for *Escherichia coli* (Mitchell and Moyle 1956), where hypothesized differences in internal osmotic pressure account for differences in desiccation resistance.

Pre-treatment of bacteria in stronger salt solutions probably increases the bacterial internal osmotic pressure and thus increases their drought resistance.

The experimental results suggest that internal osmotic pressure of the bacteria plays an important role in drought resistance.

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